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PATENT

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In re application of: Shuber

Serial No.: 08/474,450

Filed: June 7, 1995

For: **UNIVERSAL PRIMER SEQUENCE FOR MUTLIPLEX DNA AMPLIFICATION**

Examiner: J. Tung

Art Unit: 1634

APPEAL BRIEF SUBMITTED PURSUANT TO 37 CFR 1.192

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Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

This is an appeal of the Examiner's Final Rejection of pending Claims 1-18, and is submitted on or before the extended due date of April 10, 1998. Appellants hereby appeal from the Final Rejection of June 13, 1997 and the Advisory Actions of October 17, 1997 and December 23, 1997. Submitted herewith are three copies of Appellants' Appeal Brief together with a Petition for Exension of Time and the requisite fee (*see* Transmittal).

CERTIFICATE OF TRANSMISSION BY FACSIMILE

I hereby certify that this correspondence is being transmitted by facsimile machine to Examiner Gary Jones, Facsimile No. (703) 305-7939 at the United States Patent and Trademark Office on:

March 27, 1998.

Signature: Barbara Jacobs

Printed Name: Barbara Rae-Venter

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(A) Real Party of Interest

Genzyme Corporation is the real party of interest of the application at the time that the Brief is being filed.

(B) Related Appeals and Inference

No related applications are on appeal at the time that the Brief is being filed.

(C) Status of the Claims

Claims 1-18 are present in the application.

Claims 1-18 are finally rejected. Claims 1-18 are on appeal.

The text of the claims on appeal is provided in Appendix A.

Attached hereto as a separate paper is a Third Supplemental Amendment which Appellants respectfully request be entered for the reasons set forth in the amendment. The text of the proposed claims is provided in Appendix B.

(D) Status of Amendments

The Amendment in Response to the Final Rejection was entered with the exception of proposed amendments to Claims 1 and 14 containing the limitation "common sequence (oligonucleotide) not comprising a restriction enzyme recognition site sequence" (First Advisory Action mailed October 17, 1997).

The Supplemental Amendment submitted December 4, 1997 in response to the First Advisory Action mailed October 17, 1997 was not entered (Second Advisory Action mailed December 23, 1997).

(E) Summary of the Invention

Claim 1 is directed to a plurality of single-stranded oligonucleotide DNA primers for the simultaneous amplification of multiple target DNA sequences under a single set of reaction conditions in a multiplex PCR. The primers of Claim 1 comprise a 5'-X and a 3'-Y domain, wherein, the 5'-X domains share a common sequence, do not hybridize to the multiple target DNA sequences, and have a melting temperature with its complement greater than about 60°C and, wherein, the 3'-Y domains comprise unique sequences within or flanking one of the multiple target DNA sequences or its complement and have different melting temperatures. Each of the primers of Claim 1, comprising the 5'-X and a 3'-Y domains anneal specifically with its cognate target under uniform, high stringency conditions. Support for Claim 1 is found at page 3, line 17 to page 4, line 2; page 4, line 20 to page 5, line 5; page 8, line 12 to

page 9, line 13; page 10, lines 7-19; page 14, lines 3-7. Claims 2-4 are dependent claims of Claim 1. Claim 2 is supported at page 4, line 5; page 5, lines 1-2; page 8, lines 19-20; and page 12, line 15. Claim 3 is supported at page 5, lines 1-4 and page 8, lines 14-15. Claim 4 is supported at page 5, lines 4-5.

Claim 5 is directed to DNA primers for the simultaneous amplification of multiple target DNA sequences under a single set of multiplex PCR reaction conditions. The primers comprise a common 5'-sequence (X) disclosed as SEQ ID NO:64 and 3'-sequences (Y) unique to each primer that are contained within or flank one of the multiple target sequences or its complement. Support for Claim 5 is found at page 3, line 17 to page 4, line 2; page 4, line 20 to page 5, line 5; page 8, line 12 to page 9, line 13; page 10, lines 7-19; page 14, lines 3-7.

Claim 6 is directed to a method for simultaneous amplification of multiple DNA target sequences in a DNA sample in a single reaction mixture with a multiplicity of paired oligonucleotide primers having the structure, 5'-XY-3'. Each 5'-X comprises the sequence shown in SEQ ID NO:64 and each Y comprises a unique sequence within or flanking one of the multiple DNA target sequences or its complement. The multiple DNA target sequences are amplified by contacting the DNA sample-with the multiplicity of paired oligonucleotide primers and performing multiple cycles of melting, reannealing, and DNA synthesis under identical reaction conditions and cycling parameters. Support for Claim 6 is found at page 3, lines 17 to page 4, line 5; page 11, lines 15-17; page 13, lines 4-12; page 14, lines 10-18.

Claim 7 is directed to a method for simultaneously detecting multiple target DNA sequences in a DNA sample in a single reaction mixture with a multiplicity of oligonucleotide pairs consisting of first and second oligonucleotide primers. The first and second primers have the structure 5'-XY-3'. Each 5'-X comprises the sequence shown in SEQ ID NO:64 and each Y comprises a unique sequence within or flanking one of the multiple DNA target sequences or its complement. To form the amplification products for each multiple defined target DNA sequence, the multiplicity of oligonucleotide primers simultaneously contact the DNA sample in a single reaction mixture, and multiple cycles of melting, reannealing, and DNA synthesis under identical reaction conditions and cycling parameters are performed. Support for Claim 7 is found at page 4, lines 9-15; page 11, line 18 to page 12, line 2; page 13, lines 13-17; page 14, lines 19-23; page 15, lines 1-22. Claims 8 and 9 are dependent claims of Claim 7. Claim 8 is supported at page 4, lines 13-15; page 4, lines 17-18; page 9, lines 14-20; page 14, line 10 to page 16, line 5. Claim 9 is supported at page 4, lines 12-13; page 11, lines 19-21; page 14, line 10 to page 16, line 5.

Claim 10 is directed to a method for high-throughput genetic screening to simultaneously detect the presence of multiple defined target sequences in DNA samples provided by one or more individuals with a multiplicity of oligonucleotide pairs consisting of first and second oligonucleotide primers that have the structure 5'-XY-3'. Each X comprises the sequence shown in SEQ ID NO:64 and each Y is a unique sequence within one of the multiple target sequences or its complement. The sample DNA is contacted with the multiplicity of oligonucleotide pairs and subjected to multiple cycles of melting, reannealing, and DNA synthesis under the same reaction conditions and cycling parameters to form and detect the amplification products for each multiple defined target DNA sequence. Support for Claim 10 is found at page 4, lines 16-20. Claims 11 and 12 are dependent claims of Claim 10. Claim 11 is supported at page 4, lines 13-15; page 4, lines 17-18; page 9, lines 14-20; page 14, line 10 to page 16, line 5. Claim 12 is supported at page 4, lines 12-13; page 11, lines 19-21; page 14, line 10 to page 16, line 5.

Claim 13 is directed to a method to simultaneously amplify and detect multiple defined target sequences in a DNA sample by simultaneously contacting the sample with a plurality of oligonucleotide pairs consisting of first and second primers having the structure 5'-XY-3'. The X in the first and second primers has the sequence shown in SEQ ID NO:64. The Y in the first and second primers comprises unique sequences contained within one of the multiple target sequences or its complement. The sample is subjected to multiple cycles of melting, reannealing, and DNA synthesis and each cycle is conducted under the same conditions and cycling parameters, whereby, the amplification products are formed and detected. Support for Claim 13 is found at page 14, line 10 to page 16, line 5. Claim 17 is a dependent claim of Claim 13. Claim 17 is supported at page 14, line 10 to page 16, line 5.

Claim 14 is directed to a method of screening to simultaneously detect amplification products of multiple target sequences of interest in DNA by obtaining a DNA sample to be screened and contacting the sample with a plurality of oligonucleotide primer pairs having the structure 5'-XY-3' under multiplex polymerase chain reaction conditions wherein coamplification of multiple target sequences occurs in one or more cycles of identical melting, annealing and extending temperature and times and detecting the amplification products. The 5'-X domain comprises a common oligonucleotide that is neither complementary nor specific for the multiple target sequences and the 3'-Y domain comprises a unique oligonucleotide complementary to and specific for one of the multiple target sequences of interest suspected to be present in the DNA. Claim 14 is supported at page 14, line 10 to page 16, line 5. Claims 15, 16, and 18 are dependent claims of Claim 14. Claim 15 is supported at page 14, line 10 to

page 15, line 6. Claim 16 is supported at page 15, line 7 to page 16, line 5. Claim 18 is supported at page 14, line 10 to page 16, line 5.

(F) Issues

1. Whether Claims 1-5 should be rejected under 35 U.S.C § 103 as being unpatentable over Weighardt *et al.* (1993) *PCR Methods and Applications*. 3:77-80.

2. Whether Claims 6-18 should be rejected under 35 U.S.C § 103 as being unpatentable over Picci *et al.* (1992) *Hum. Genet.* 88:552-556 in view of Weighardt *et al.*

3. Whether Claims 17 and 18 should be rejected under 35 U.S.C § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

(G) Grouping of Claims

Each of Claims 1-18 stand alone; the rejected claims do not stand or fall together. Reasons as to why Appellants consider the rejected claims to be separately patentable are included in paragraph (H) below in the Arguments relating to the individual rejections.

(H) Arguments

1. Entry of Amendments

Entry of the claim amendments would resolve the 35 U.S.C. § 103 issues on appeal because the amended claims would more precisely define the claimed invention and the unexpected advantages it provides over the prior art. Entry of the claim amendments also would resolve the 35 U.S.C. § 112, second paragraph issue on appeal by placing Claims 17 and 18 in independent form. Through the recitation of language of the claims from which they previously depended, Claims 17 and 18 distinctly define the subject matter of the claimed invention.

2. Claims 1-5 are patentable over Weighardt *et al.*

To simplify the discussion, the separate elements of each claim are provided below.

Claim 1, has the following elements:

Element 1. A plurality of single-stranded oligonucleotide DNA primers for simultaneous amplification of multiple target DNA sequences

under a single set of reaction conditions in a multiplex polymerase chain reaction (PCR), said primers having a

Element 2. 5' domain, X, wherein a) said 5'-X domains each comprise a common sequence that does not hybridize to said multiple target sequences,

Element 3. 3' domain, Y, wherein c) said 3'-Y domains each comprise a unique sequence contained within or flanking one of said multiple target sequences or its complement;

Element 4. b) the melting temperature of a hybrid between X and its complement in the absence of other sequences is greater than about 60°C;

Element 5. d) the melting temperature of a hybrid between at least one of said 3'-Y domains and its complement, in the absence of other sequences, is different from the melting temperature of a hybrid between at least one other 3'-Y domain and its complement present in said multiplex PCR; and

Element 6. each of said primers being capable of annealing specifically with its cognate target sequence under uniform high stringency annealing conditions during said amplification.

Claims 2-4 are dependent *inter alia* upon Claim 1, and thus include each of the elements of Claim 1. The dependent claims have the indicated additional limitation: Claim 2, the common sequence in Element 2 is 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64). Claim 3, the common sequence of Element 2 and the unique sequence of Element 3 each comprise 17-20 bases; Claim 4, an additional element is introduced:

Element 7. the melting temperature of a hybrid formed between each of said primers and its complement in a solution of 0.5 M NaCl is at least 72°C.

The § 103 rejection of Claim 1 over Weighardt *et al.* is erroneous because the reference does not disclose or suggest Elements 1, 2, 4, 5, and 6 of Claim 1 or Element 7 of Claim 4.

Element 1 of Claim 1 recites a plurality, or more than one set, of single-stranded oligonucleotide DNA primers. Weighardt *et al.* discloses a single set of single-stranded oligonucleotide DNA primers.

Element 2 of Claim 1 recites that the 5'-X domains of each primer do not hybridize to the multiple target sequences. Weighardt *et al.* describe primers in which the 5'-domain is "an unrelated sequence" and provides no further description. This description does not teach one of skill in the art to use sequences that *do not hybridize* to the target sequence, because sequences that are unrelated by statistical analysis may still share sufficient sequence homology to hybridize to target sequences. Non-specific hybridization is a problem commonly associated with oligonucleotides. This is especially true when a region of homology is concentrated in a single area, as is the case in Weighardt *et al.* The 5'-domains of Weighardt *et al.* contain restriction endonuclease cleavage sites. These sites are commonly found in DNA sequences and therefore, promote relatedness and hybridization between the 5'-domain of the primer and the target sequence. Weighardt *et al.* provide no teaching to avoid homology between the 5'-domain and the target DNA. To avoid the use of a restriction endonuclease site in the 5'-domain of the primers that also may be present in the target DNA sequence would require undue experimentation because it requires analysis of the target DNA by either random restriction enzyme digestion, the random synthesis and testing of primers with different restriction site sequences or sequencing the DNA target in its entirety. This type of experimentation is undue because the target DNA may not be available in sufficient amounts to permit these types of analyses.

Element 5 of Claim 1 recites that the melting temperature of a hybrid between at least one of the 3'-Y domains and its complement, in the absence of other sequences, is different from the melting temperature of a hybrid between at least one other 3'-Y domain and its complement, in the absence of other sequences. In other words, the melting temperature of each individual Y-domain can be unique. The melting temperatures of the individual Y-domains does not need to be uniform. Element 4 of Claim 1 states that the melting temperature of a hybrid between X and its complement in the absence of other sequences is greater than about 60°C. The melting temperature of the X-domains must, therefore, be uniform but must also be above a specific threshold. This a critical inventive step because it is the X-domain that enables the entire primer, 5'-XY-3', to anneal specifically with its cognate target sequence under uniform high stringency

conditions (*see* Element 6). The X-domain imposes on the entire primer (5'-XY-3') the ability to anneal under uniform high stringency. This is not a property of the Y-domains because as stated in Element 5, their melting temperatures are not uniform. Weighardt *et al.* provides no teaching with respect to these aspects of the claimed invention and, therefore, the primers of Weighardt *et al.* do not render the primers of the claimed invention obvious.

Element 6 of Claim 1 states that each primer anneals specifically with its cognate target sequence under uniform high stringency conditions during amplification. This contrasts with the primers disclosed by Weighardt *et al.* which require individual extensions under low stringency at T_1 and T_2, prior to high stringency amplification (Figure 2, page 78). Weighardt *et al.* defines these preliminary steps as "extensions" but they are preliminary amplifications conducted under low stringency. The requirement for low stringency amplification indicates that the primers of Weighardt *et al.* do not anneal with their cognate target sequences under uniform high stringency conditions. Therefore, Weighardt *et al.* teach away from the claimed invention.

Claims 2-4 are dependent from Claim 1 and therefore also are patentable over Weighardt *et al.* Furthermore, each claim recites an additional limitation not disclosed by Weighardt *et al.* Claim 2 recites a nucleotide sequence of the 5'-X domain (Element 2), 5'-GCGGTCCCAAAAGGGTCAGT (SEQ ID NO:64). The 5'-X domain taught by Weighardt *et al.*, 5'-GGCATAGCTGAATGCAT, is not homologous to the sequence recited in Claim 2 and does not render the claimed invention obvious. Claim 3 recites that the common sequence of Element 3 of Claim 1 is 17-20 bases in length. In contrast, Weighardt *et al.* teaches an oligonucleotide common sequence of about 10-15 nucleotides, althought there is an example of a specific 5'-X domain of 17 bases.

Element 7 recited in Claim 4 states that the melting temperature (T_m) of a hybrid formed between each of the primers (5'-XY-3') and its complement in a solution of 0.5 M NaCl is at least 72°C. The T_m of oligonucleotides is influenced by salt concentration or ionic strength. In solutions of moderate ionic strength (0.05 M KCl, 0.0015 M $MgCl_2$), conditions under which enzyme reactions occur, hybridization between oligonucleotides is enhanced and the T_m increases because the cations neutralize the negative charge of the DNA's phosphate backbone that would otherwise inhibit hybridization between complementary DNA molecules. As the ionic strength increases to very high levels, hybridization and the T_m decrease because the ions disrupt hydrogen bonding (electrostatic interactions) required for base pairing between complementary

DNA molecules. Weighardt *et al.* also teach that their entire primer should have a T_m of 72°C or greater but do not state the conditions under which a T_m greater than 72°C should be achieved. Because the PCR of Weighardt *et al.* uses 72°C as the annealing temperature, it can be assumed that the T_m is measured under normal PCR conditions or moderate ionic strength. Claim 4 specifically recites a T_m of greater than 72°C in 0.5 M NaCl, which is a relatively high ionic strength solution in comparison to normal PCR conditions (0.05 M KCl, 0.0015 M $MgCl_2$; page 13, lines 6-7). Therefore, the primers of Claim 4 have a significantly higher affinity for their cognate target sequences than those disclosed by Weighardt *et al.* Therefore, Weighardt *et al.* teach away from Element 7 of Claim 4.

Claim 5, has the following elements:

Element 1. DNA primers for simultaneous amplification of multiple target DNA sequences under a single set of reaction conditions in a multiplex polymerase chain reaction (PCR), wherein

Element 2. said primers consist of the sequence 5'-GCGGTCCCAAAAGGGTCGT (SEQ ID NO:64) (Y)-3', wherein

Element 3. an individual Y comprises a unique sequence contained within or flanking one of said multiple target sequences or its complement.

The § 103 rejection of Claim 5 over Weighardt *et al.* is erroneous because the reference does not disclose or suggest Element 2.

Element 2 of Claim 5 recites that the primers consist of the sequence 5'-GCGGTCCCAAAAGGGTCGT (SEQ ID NO:64) (Y)-3'. The primers of Weighardt *et al.* consist of the sequence 5'-GGCATAGCTGAATGCAT (Y)-3' which has no sequence homology to the claimed Element and, therefore, can not render it obvious. Claim 5, therefore, is patentable over Weighardt *et al.*

In summary, the composition and characteristics of the primers of Weighardt *et al.* are, fundamentally different from the claimed primers. In addition, the primers of Weighardt *et al.* contain a restriction endonuclease recognition site. The presence of a restriction endonuclease recognition site conflicts with the requirement of the 5'-domain of the claimed invention as being unable to hybridize to the target DNA sequences (Claim 1, Element 4) and also conflicts with the description of Weighardt *et al.* of the 5'-domain as being "unrelated" to the template DNA.

Weighardt *et al.* also do not describe a threshold melting temperature for the X-domain (Element 4, Claim 1), that the melting temperatures of the Y-domain do not need to be uniform (Element 5, Claim 1), or that the melting temperature of the entire primer 5'-XY-3' in 0.5 M NaCl is at least 72°C. The sequence of the 5'-domain of Weighardt *et al.* also is unrelated to the 5'-domain of the claimed primers (Claim 2). In the absence of a hindsight analysis of Weighardt *et al.* it would have required random, undue experimentation to arrive at the 5'-domain sequence of the claimed invention based on the teaching of Weighardt *et al.*

3. Claims 6-18 are patentable over Picci *et al.* (1992) *Hum. Genet.* 88:552-556 in view of Weighardt *et al.*

To simplify the discussion, the separate elements of each claim are provided below.

Claim 6, has the following elements:

Element 1. A method for simultaneous amplification of multiple DNA target sequences present in a DNA sample, said method comprising:

Element 2. a) contacting said DNA sample, in a single reaction mixture, with a multiplicity of paired oligonucleotide primers having structure 5'-XY-3', wherein

Element 3. (i) each X comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT (SEQ ID NO:64), and

Element 4. (ii) each Y comprises a unique sequence contained within or flanking one of said multiple target sequences or its complement; and

Element 5. b) performing multiple cycles of melting, reannealing, and DNA synthesis under identical reaction conditions and cycling parameters.

Claim 7, has the following elements:

Element 1. A method of simultaneously detecting the presence of multiple defined target DNA sequences in a DNA sample, which comprises the steps of:

Element 2. a) simultaneously contacting said DNA, in a single reaction mixture, with a multiplicity of oligonucleotide pairs,

Element 3. each of said pairs consisting of a first and a second oligonucleotide primer, wherein

Element 4. (i) said first primer of each pair has the structure 5'-XY-3', wherein

Element 5. each X comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64) and

Element 6. each Y comprises a unique sequence contained within or flanking one of said multiple target sequences or its complement, and

Element 7. (ii) said second primer of each pair has the structure 5'-XY-3', wherein

Element 8. each X comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64) and

Element 9. each Y comprises a unique sequence contained within or flanking one of said multiple target sequences or its complement;

Element 10. b) performing multiple cycles of melting, reannealing, and DNA synthesis under identical reaction conditions and cycling parameters to form amplification products for each of said multiple defined target DNA sequences primed with said oligonucleotide; and

Element 11. c) detecting the amplification products.

Claims 8 and 9 are dependent *inter alia* upon Claim 7, and thus include each of the elements of Claim 7. The dependent claims have the indicated additional limitation: Claim 9, the detecting of amplification products of Element 11 comprises gel electrophoresis and Claim 8, an additional element is introduced:

Element 12. the detection of amplification products indicates the presence of the target sequence in a DNA sample.

Claim 10, has the following elements:

Element 1. A method for high-throughput genetic screening to simultaneously detect the presence of multiple defined target sequences in DNA

samples obtained from one or more individuals, said method comprising steps of:

Element 2. a) providing a sample of DNA from said individual(s);

Element 3. b) simultaneously contacting said DNA sample(s) with a multiplicity of oligonucleotide pairs,

Element 4 each of said pairs consisting of a first and a second oligonucleotide primer, wherein

Element 5. (i) said first primer of each pair has the structure 5'-XY-3', wherein

Element 6. each X comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64) and

Element 7. each Y comprises a unique sequence contained within or flanking one of said multiple target sequences or its complement, and

Element 8. (ii) said second primer of each pair has the structure 5'-XY-3', wherein

Element 9. each X comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64) and

Element 10. each Y comprises a unique sequence contained within or flanking one of said multiple target sequences or its complement;

Element 11. c) subjecting said sample to multiple cycles of melting, reannealing, and DNA synthesis wherein,

Element 12. each of said cycles is conducted under the same reaction conditions and cycling parameters to form amplification products for each of said multiple defined target DNA sequences primed with said oligonucleotide; and

Element 13. d) detecting the amplification products.

Claims 11 and 12 are dependent *inter alia* upon Claim 10, and thus include each of the elements of Claim 10. The dependent claims have the indicated additional limitation: Claim 12, detecting of amplification products of Element 11 comprises gel electrophoresis; and Claim 11, an additional element is introduced:

Element 14. the detection of amplification products indicates the presence of the target sequence in the DNA sample.

Claim 13, has the following elements:

Element 1. A method for simultaneously amplifying and detecting multiple defined target sequences in a DNA sample, said method comprising steps of:

Element 2. a) simultaneously contacting said sample with a plurality of oligonucleotide pairs, each of said pairs consisting of

Element 3. a first and a second primer having the structure 5'-XY-3', wherein,

Element 4 (i) X in said first primer of each pair comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64) and

Element 5. Y comprises a unique sequence contained within one of said multiple target sequences or its complement, and

Element 6. (ii) said second primer of each pair has the structure 5'-XY-3', wherein,

Element 7. each X comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64) and

Element 8. each Y comprises a unique sequence flanking one of said multiple target sequences or its complement;

Element 9. c) subjecting said sample to multiple cycles of melting, reannealing, and DNA synthesis wherein,

Element 10. each of said cycles is conducted under the same reaction conditions and cycling parameters to form amplification products for each of said multiple defined target sequences primed with said oligonucleotide; and

Element 11. d) detecting the amplification products.

Claim 17 is a product by process claim, in which the claimed product is made by the process of Claim 13, and thus includes each of the elements of Claim 13.

Element 2

Element 2. A plurality of amplified target sequences of interest amplified and detected according to the method of Claim 13.

Claim 14. has the following elements:

Element 1. A method of screening to simultaneously amplifying and detect multiple target sequences of interest in DNA, the method comprising:

Element 2. a) obtaining a sample of DNA to be screened for said multiple target sequences of interest,

Element 3. b) contacting said sample with a plurality of oligonucleotide primer pairs having the structure 5'-XY-3' under multiplex polymerase chain reaction conditions wherein,

Element 4. coamplification of multiple target sequences occurs in one or more cycles of identical melting, annealing and extending temperatures and times, wherein

Element 5. each 5'-X domain comprises a common oligonucleotide that is

Element 6. neither complementary nor specific for said multiple target sequences,

Element 7. each 3'-Y domain comprises a unique oligonucleotide,

Element 8. each oligonucleotide complementary to and specific for one of said multiple target sequences of interest suspected to be present in said DNA; and

Element 9. c) detecting the amplification products.

Claims 15, 16, and 18 are dependent *inter alia* upon Claim 14, and thus include each of the elements of Claim 14. The dependent claims have the indicated additional limitations: Claim 15, the multiple target sequences of interest of Element 1 are located within different regions of a gene present in said DNA; Claim 16, the multiple target sequences of interest of Element 1 are located within multiple genes present in DNA.

Claim 18, is a product by process claim in which the claimed product is made by the process according to Claim 14.

Element 2

Element 2. A plurality of amplified target sequences of interest amplified and detected according to the method of Claim 14.

The § 103 rejection of Claims 6-18 over Picci *et al.* in view of Weighardt *et al.* is erroneous because neither reference, individually or in combination, discloses or suggests all of the elements of each claim. Picci *et al.* teach away from the claimed invention by disclosing a method that requires an incubation step prior to PCR and that employ primers that do not share a common 5'-domain. Weighardt *et al.* teach away from the claimed invention by disclosing methods comprising a multiple set of reaction conditions for the amplification of a single target DNA.

Claim 6, Element 3; Claim 7, Elements 5 and 8; Claim 10, Elements 6 and 9; and Claim 13, Elements 4 and 7 each recite a 5'-X domain comprising the nucleotide sequence (5'-GCGGTCCCAAAAGGGTCAGT (SEQ ID NO:64)). Weighardt *et al.* teach a 5'-domain of primers having the sequence 5'-GGCATAGCTGAATGCAT which has no homology to the claimed invention and, therefore, does not render the claimed invention obvious.

Claim 6, Element 5; Claim 7, Element 10; Claim 10, Element 12; Claim 13, Element 10; and Claim 14, Element 4 each recite performing multiples PCR cycles under identical or the same set of reaction conditions. The claimed methods do not require preliminary incubation or extension steps and can be summarized as shown in Figure 1.

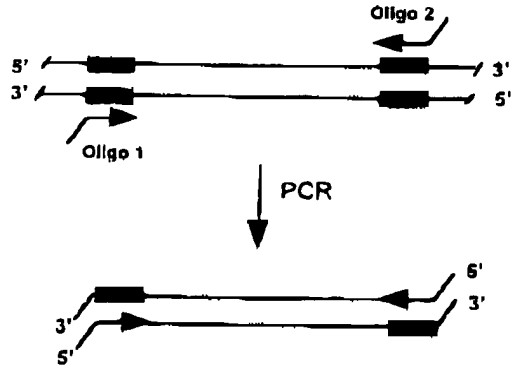

Figure 1

Picci *et al.* teach a multiplex PCR method that requires heating the reaction to 94°C for 5 minutes prior to multiplex PCR, a step not required by the claimed method. The further

addition of Weighardt *et al.* does not cure the deficiencies of Picci *et al.* The first method disclosed by Weighardt *et al.* consists of three steps: i) the addition of oligonucleotide 1 with the lower annealing temperature (T_1) and about five extensions at T_1, ii) the addition of oligonucleotide 2 and five PCR cycles performed at T_2 ($T_2 > T_1$), and iii) PCR. The second method also consists of three steps: i) the simultaneous addition of oligonucleotide 1 and oligonucleotide 2 and one extension at T_1, ii) five PCR cycles performed at T_2 ($T_2 > T_1$), and iii) PCR (see Figure 1, page 78).

Further distinctions between Picci *et al.* and Weighardt *et al.* are addressed in the Third Supplemental Amendment attached hereto. In the method of Picci *et al.*, the primers formed dimers that resulted in the generation of unwanted, additional products (page 553, right column). This deficiency of Picci *et al.* would not be cured by the primers of Weighardt *et al.* The method of Weighardt *et al.* requires individual extension reactions for each primer prior to PCR (Figure 1, page 78) and Picci *et al.* do not disclose individual extension reactions prior to PCR. Without the individual extension reactions, Weighardt *et al.* state on page 78, right column, lines 7-12 and 40-44 and page 79, Figure 2 legend that very little of the expected product is produced and it is contaminated by a great number of unwanted, spurious fragments. Furthermore, the primers of Weighardt *et al.* have restriction endonuclease sites in the 5'-domain, that because of their palindromic sequence, would promote primer-dimer formation and the generation of unwanted, spurious products when used in the method of Picci *et al.* The primers of Weighardt *et al.* contain an *Nsi* I restriction endonuclease site: 5'-ATGCAT-3' (Figure 2, legend). Because this sequence is palindromic it can hybridize to the identical sequence present in the 5'-domain of another primer:

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Primer 1:  5'-...ATGCAT...-3'
                  ||||||
Primer 2:  3'-...TACGTA...-5'.
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The restriction endonuclease site will, therefore, promote the formation of primer-dimers which can serve as a substrate for DNA polymerase resulting in the formation of unwanted, spurious PCR products. Weighardt *et al.* do not cure the deficiencies of Picci *et al.* but rather would enhance these deficencies by promoting primer-dimer formation. Therefore, the skilled artisan would not have been motivated to combine these two references because there would have been no expectation of success.

This contrasts with the claimed methods, wherein, steps (i) and (ii) of Weighardt *et al.* are not required. Furthermore, in the methods disclosed by Weighardt *et al.* only a single target

DNA sequence is amplified. This is in contrast to Claim 6, Element 1; Claim 7, Element 1; Claim 10, Element 1; Claim 13, Element 1; and Claim 14, Element 1 that recite "the simultaneous amplification of multiple target sequences".

Claim 16 discloses the additional limitation of the multiple target sequences of Claim 14, Element 1 as being located within multiple genes present in the DNA sample and is patentable over Picci *et al.* and Weighardt. Picci *et al.* does not disclose a method for amplifying target sequences which are located within multiple genes and Weighardt *et al.* does not disclose a method for amplifying multiple target sequences.

Claims 17 and 18 are patentable over Picci *et al.* and Weighardt *et al.* because neither reference discloses the plurality of amplified target sequences of the claimed invention. Weighardt *et al.* does not disclose a plurality of amplified sequences. Weighardt *et al.* is limited to amplifying a single target sequence. Picci *et al.* discloses four amplified target sequences from the cystic fibrosis transmembrane regulator (CFTR) gene; however, Picci *et al.* state on page 553, right column that their amplified sequences are contaminated by spurious bands produced by primer-dimers. The claimed invention discloses by working exemplification on on page 14, line 23 to page 15, line 2, a plurality of amplified sequences of the CFTR gene that "are virtually free of contaminating non-specific products." Further distinctions between the claimed invention and Picci *et al.* are addressed in the Third Supplemental Amendment attached hereto, wherein. Claims 17 and 18 have been amended to recite "free of spurious amplification products."

In summary, neither reference either individually or in combination suggests all the elements of Claims 6-18 and no convincing line of reasoning has been provided as to why the artisan would have found the claimed methods to have been obvious in light of these references.

4. Claims 17 and 18 are definite in pointing out the subject matter of the claimed invention.

Claims 17 and 18 have been rejected under § 112, second paragraph as being indefinite by reciting "a plurality of amplified target sequences of interest" and because it cannot be determined from the specification what is encompassed by a plurality of nucleic acids defined only by how the nucleic acids are detected.

This rejection is erroneous because the term "a plurality of amplified target sequences of interest" is defined in the specification and the plurality of amplified target sequences of interest

of Claims 17 and 18 are not defined by how they are detected. Claims 17 and 18 are product by process claims, wherein, a product, a plurality of amplified target sequences of interest, is defined in terms of the process by which it is made and detected. The processes are defined in Claims 13 and 14 from which Claims 17 and 18, respectively, depend. The term, "a plurality of amplified target sequences of interest", produced by the processes of Claims 13 and 14 is defined in the specification at page 7, lines 11-13. By way of exemplification, the specification discloses a plurality of amplified target sequences of the cystic fibrosis transmembrane regulator (CFTR) gene in Example 2, page 14, line 22 to page 15, line 2. Additional examples are found at page 15, line 7 to page 17, line 5, wherein, a plurality of target sequences of the CFTR, alpha-galactosidase, sickle-cell, Tay-Sachs, beta-thalassemia and WT-1 genes as defined by Claims 17 and 18 are disclosed. Therefore, it would be clear to one of skill in the art what is encompassed by "a plurality of amplified target sequences of interest" as recited in Claims 17 and 18 and to differentiate them from a plurality of amplified target sequences produced by other methods.

CONCLUSION

Based upon the above arguments, it is submitted that Claims 1-18 are allowable and the honorable Board is respectfully requested to reverse the Examiner's rejections and remand the application for issue.

Three copies of the Appeal Brief are provided along with payment of the required fee.

Respectfully submitted,

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APPENDIX A

CLAIMS ON APPEAL

1. A plurality of single-stranded oligonucleotide DNA primers for simultaneous amplification of multiple target DNA sequences under a single set of reaction conditions in a multiplex polymerase chain reaction (PCR), said primers having a 5' domain, X, and a 3' domain, Y, wherein

a) said 5'-X domains each comprise a common sequence that does not hybridize to said multiple target sequences;

b) the melting temperature of a hybrid between X and its complement in the absence of other sequences is greater than about 60°C;

c) said 3'-Y domains each comprise a unique sequence contained within or flanking one of said multiple target sequences or its complement;

d) the melting temperature of a hybrid between at least one of said 3'-Y domains and its complement, in the absence of other sequences, is different from the melting temperature of a hybrid between at least one other 3'-Y domain and its complement present in said multiplex PCR; and

each of said primers being capable of annealing specifically with its cognate target sequence under uniform high stringency annealing conditions during said amplification.

2. The primers according to claim 1, wherein X comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64).

3. The primers according to claim 1, wherein X and Y each comprise from 17 to 20 bases.

4. The primers according to claim 1, wherein the melting temperature of a hybrid formed between each of said primers and its complement in a solution of 0.5 M NaCl is at least 72°C.

5. DNA primers for simultaneous amplification of multiple target DNA sequences under a single set of reaction conditions in a multiplex polymerase chain reaction (PCR), wherein said primers consist of the sequence 5'-GCGGTCCCAAAAGGGTCGT (SEQ ID NO:64) (Y)-3'.

wherein an individual Y comprises a unique sequence contained within or flanking one of said multiple target sequences or its complement.

6. A method for simultaneous amplification of multiple DNA target sequences present in a DNA sample, said method comprising:

a) contacting said DNA sample, in a single reaction mixture, with a multiplicity of paired oligonucleotide primers having structure 5'-XY-3', wherein

(i) each X comprises the sequence 5'- GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64), and

(ii) each Y comprises a unique sequence contained within or flanking one of said multiple target sequences or its complement; and

b) performing multiple cycles of melting, reannealing, and DNA synthesis under identical reaction conditions and cycling parameters.

7. A method for simultaneously detecting the presence of multiple defined target DNA sequences in a DNA sample, which comprises the steps of:

a) simultaneously contacting said DNA sample, in a single reaction mixture, with a multiplicity of oligonucleotide pairs, each of said pairs consisting of a first and a second oligonucleotide primer, wherein

(i) said first primer of each pair has the structure 5'-XY-3', wherein each X comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64) and each Y comprises a unique sequence contained within one of said multiple target sequences or its complement, and

(ii) said second primer of each pair has the structure 5'-XY-3', wherein each X comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64), and each Y comprises a unique sequence flanking one of said multiple target sequences or its complement

b) performing multiple cycles of melting, reannealing, and DNA synthesis under identical reaction conditions and cycling parameters to form amplification products for each of said multiple defined target DNA sequences primed with said oligonucleotide; and

c) detecting the amplification products.

8. The method of claim 7 wherein detection of amplification product indicates the presence of the target sequence in a DNA sample.

9. The method of claim 7 wherein said detecting step comprises gel electrophoresis.

10. A method for high-throughput genetic screening to simultaneously detect the presence of multiple defined target sequences in DNA samples obtained from one or more individuals, said methods comprising steps of:

a) providing a sample of DNA from said individual(s);

b) simultaneously contacting said DNA sample(s) with a multiplicity of oligonucleotide pairs, each of said pairs consisting of a first and second oligonucleotide primer, wherein

(i) said first primer of each pair has the structure 5'-XY-3', wherein each X comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64) and each Y comprises a unique sequence contained within one of said multiple target sequences or its complement, and

(ii) said second primer of each pair has the structure 5'-XY-3', wherein each X comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64), and each Y comprises a unique sequence flanking one of said multiple target sequences or its complement;

c) subjecting said sample to multiple cycles of melting, reannealing, and DNA synthesis wherein each of said cycles is conducted under the same reaction conditions and cycling parameters to form amplification products for each of said multiple defined target DNA sequences primed with said oligonucleotides; and

d) detecting the amplification products.

11. The method of claim 10 wherein detection of an amplification product indicates the presence of the target sequence in the DNA sample.

12. The method of claim 10 wherein said detecting step comprises gel electrophoresis.

13. A method for simultaneously amplifying and detecting multiple defined target sequences in a DNA sample, said method comprising the steps of:

a) simultaneously contacting said sample with a plurality of oligonucleotide pairs, each of said pairs consisting of a first and a second primer having the structure 5'-XY-3', wherein

(i) X in said first primer of each pair comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64) and Y comprises a unique sequence contained within one of said multiple target sequences or its complement, and

(ii) said second primer of each pair as the structure 5'-XY-3', wherein each X comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64), and each Y comprises a unique sequence flanking one of said multiple target sequences or its complement;

c) subjecting said sample to multiple cycles of melting, reannealing, and DNA synthesis wherein each of said cycles is conducted under the same conditions and cycling parameters to form amplification products for each of said multiple defined target sequences primed with said oligonucleotides, and

d) detecting the amplification products.

14. A method of screening to simultaneously amplify and detect multiple target sequences of interest in DNA, the method comprising:

a) obtaining a sample of DNA to be screened for said multiple target sequences of interest,

b) contacting said sample with a plurality of oligonucleotide primer pairs having the structure 5'-XY-3' under multiplex polymerase chain reaction conditions wherein coamplification of said multiple target sequences occurs in one or more cycles of identical melting, annealing and extending temperatures and times, wherein

each 5'-X domain comprises a common oligonucleotide that is neither complementary nor specific for said multiple target sequences; and

each 3'-Y domain comprises a unique oligonucleotide, each oligonucleotide complementary to and specific for one of said multiple target sequences of interest suspected to be present in said DNA; and

c) detecting the amplification products.

15. A method according to claim 14, wherein said multiple target sequences of interest are located within different regions of a gene present in said DNA.

16. A method according to claim 14, wherein said multiple target sequences of interest are located within multiple genes present in said DNA.

17. A plurality of amplified target sequences of interest amplified and detected according to the method of claim 13.

18. A plurality of amplified target sequences of interest amplified and detected according to the method of claim 14.

APPENDIX B

PROPOSED AMENDED CLAIMS

1. A multiplicity of single-stranded oligonucleotide DNA primers for simultaneous amplification of multiple target DNA sequences under a single set of reaction conditions in a single multiplex polymerase chain reaction (PCR), said primers having a 5' X domain and a 3' Y domain, wherein;

a) each said 5' X domain comprises a common sequence that does not hybridize to and has no homology with any one of said multiple target DNA sequences or its complement, whereby the synthesis of spurious amplification products are prevented;

b) the melting temperature of a hybrid between X and its complement in the absence of other sequences is greater than about 60°C;

c) each said 3'Y domain comprises a unique sequence contained within or flanking one of said multiple target DNA sequences or its complement whereby the synthesis of spurious amplification products are prevented; and

d) the melting temperature of a hybrid between at least one of said 3'-Y domains and its complement, in the absence of other sequences, is different from the melting temperature of a hybrid between at least one other 3'-Y domain and its complement present in said multiplex PCR; and

e) each of said primers being capable of annealing specifically with its cognate target sequence under uniform high stringency annealing conditions during said amplification.

2. The multiplicity of single-stranded oligonucleotide DNA primers according to claim 1, wherein X comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64).

3. The multiplicity of single-stranded oligonucleotide DNA primers according to claim 1, wherein X and Y each comprise from 17 to 20 bases.

4. The multiplicity of single-stranded oligonucleotide DNA primers according to claim 1, wherein the melting temperature of a hybrid formed between each of said primers and its complement in a solution of 0.5M NaCl is at least 72°C.

5. A multiplicity of single-stranded oligonucleotide DNA primers for simultaneous amplification of multiple target DNA sequences under a single set of reaction conditions in a single multiplex polymerase chain reaction (PCR), wherein said primers consist of the sequence 5'-GCGGTCCCAAAAGGGTCGT (SEQ ID NO:64) (Y)-3', wherein an individual Y comprises a unique sequence contained within or flanking one of said multiple target DNA sequences or its complement.

6. A method for simultaneous amplification of multiple target DNA sequences present in a DNA sample, said method comprising:

a) contacting said DNA sample, in a single reaction mixture, with a multiplicity of single-stranded oligonucleotide DNA primer pairs having a 5' X domain, and a 3'Y domain, wherein

(i) each said X domain comprises the common sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64), whereby the synthesis of spurious amplification products are prevented, and

(ii) each said Y domain comprises a unique sequence contained within or flanking one of said multiple target sequences or its complement, whereby the synthesis of spurious amplification products are prevented; and

b) performing multiple cycles of melting, reannealing, and DNA synthesis under identical reaction conditions and cycling parameters.

7. A method for simultaneously detecting the presence of multiple target DNA sequences in a DNA sample, which comprises the steps of:

a) simultaneously contacting said DNA sample, in a single reaction mixture, with a multiplicity of single-stranded oligonucleotide DNA primer pairs, each of said multiplicity of single-stranded oligonucleotide DNA primer pairs consisting of a first oligonucleotide DNA primer and a second oligonucleotide DNA primer, wherein

(i) said first oligonucleotide DNA primer has a 5' X domain and a 3'Y domain, wherein each said X domain comprises the common sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64) and each said Y domain comprises a unique sequence contained within or flanking one of said multiple target DNA sequences or its complement, and

(ii) said second oligonucleotide DNA primer has a 5' X domain and a 3'Y domain, wherein each said X domain comprises the common sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64), and each said Y domain comprises a

unique sequence contained within or flanking one of said multiple target DNA sequences or its complement,

whereby the synthesis of spurious amplification products are prevented; and

b) performing multiple cycles of melting, reannealing, and DNA synthesis under identical reaction conditions and cycling parameters to form amplification products for each of said multiple target DNA sequences amplified with said multiplicity of single-stranded oligonucleotide DNA primers; and

c) detecting said amplification products.

8. The method according to Claim 7, wherein detection of said amplification products indicates the presence of said multiple target DNA sequences in said DNA sample.

9. The method according to Claim 7, wherein said step of detecting comprises gel electrophoresis.

10. A method for high-throughput genetic screening to simultaneously detect the presence of multiple target DNA sequences in DNA sample(s) obtained from one or more individuals, said method comprising the steps of:

a) simultaneously contacting said DNA sample(s) with a multiplicity of single-stranded oligonucleotide DNA primer pairs, each of said pairs consisting of a first oligonucleotide DNA primer and a second oligonucleotide DNA primer, wherein

(i) said first oligonucleotide DNA primer of each pair has a 5' X domain and a 3'Y domain, wherein each X domain comprises the common sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64) and each Y domain comprises a unique sequence contained within or flanking one of said multiple target DNA sequences or its complement, and

(ii) said second primer of each pair has a 5' X domain and a 3'Y domain, wherein each X domain comprises the common sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64), and each Y domain comprises a unique sequence contained within or flanking one of said multiple target sequences or its complement,

whereby the synthesis of spurious amplification products are prevented.

b) subjecting said sample to multiple cycles of melting, reannealing, and DNA synthesis wherein each of said cycles is conducted under the same reaction conditions and cycling parameters to form amplification products for each of said multiple target DNA sequences; and

c) detecting said amplification products.

11. The method according to Claim10, wherein detection of said amplification products indicates the presence of said multiple target DNA sequence(s) in said DNA sample(s).

12. The method according to Claim 10, wherein said step of detecting comprises gel electrophoresis.

13. A method for simultaneously detecting amplification products of multiple target DNA sequence(s) in a DNA sample(s), said method comprising the steps of:

a) simultaneously contacting said DNA sample(s) with a multiplicity of single-stranded oligonucleotide DNA primer pairs, each of said pairs consisting of a first oligonucleotide DNA primer and a second oligonucleotide DNA primer each having a 5' X domain and a 3'Y domain, wherein

(i) said X domain in said first oligonucleotide DNA primer comprises the common sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64) and said Y domain comprises a unique sequence contained within or flanking one of said multiple target DNA sequences or its complement, and

(ii) said X domain in said second oligonucleotide DNA primer comprises the common sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64), and said Y domain comprises a unique sequence contained within or flanking one of said multiple target DNA sequences or its complement;

c) subjecting said sample(s) to multiple cycles of melting, reannealing, and DNA synthesis wherein each of said cycles is conducted under the same conditions and cycling parameters to form amplification products for each of said multiple target DNA sequences primed with said oligonucleotides, and

d) detecting said amplification products.

14. A method of screening to simultaneously detect amplification products of multiple target DNA sequences in DNA sample(s), said method comprising the steps of:

a) contacting said DNA sample(s) with a multiplicity of single-stranded oligonucleotide DNA primer pairs having a 5'X domain, and a 3'Y domain, under single multiplex polymerase chain reaction conditions wherein coamplification of said multiple target DNA

sequences occurs in one or more cycles of identical melting, annealing and extending temperatures and times, wherein

each said X domain comprises a common sequence that is neither complementary to nor specific for said multiple target DNA sequences, whereby the synthesis of spurious amplification products are prevented; and

each said Y domain comprises a unique sequence, wherein said unique sequence is complementary to and specific for one of said multiple target DNA sequences suspected to be present in said DNA sample(s), whereby the synthesis of spurious amplification products are prevented; and

b) detecting said amplification products.

15. The method according to Claim 14, wherein said multiple target DNA sequences are located within different regions of a gene present in said DNA sample(s).

16. The method according to Claim 14, wherein said multiple target DNA sequences are located within multiple genes present in said DNA sample(s).

17. A multiplicity of amplified target DNA sequences free of spurious amplification products produced according to the method of

a) simultaneously contacting a DNA sample(s) with a multiplicity of single-stranded oligonucleotide DNA primer pairs, each of said pairs consisting of a first oligonucleotide DNA primer and a second oligonucleotide DNA primer each having a 5'X domain and a 3'Y domain, wherein

(i) said X domain in said first oligonucleotide DNA primer comprises the common sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64) and said Y domain comprises a unique sequence contained within or flanking one of said multiple target DNA sequences or its complement, and

(ii) said X domain in said second oligonucleotide DNA primer comprises the common sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64), and said Y domain comprises a unique sequence contained within or flanking one of said multiple target DNA sequences or its complement;

c) subjecting said sample(s) to multiple cycles of melting, reannealing, and DNA synthesis wherein each of said cycles is conducted under the same conditions and cycling parameters, whereby a multiplicity of amplified target DNA sequences are obtained.

18. A multiplicity of amplified target DNA free of spurious amplification products produced according to the method of contacting a DNA sample(s) with a multiplicity of single-stranded oligonucleotide DNA primer pairs having a 5'X domain, and a 3'Ydomain, under single multiplex polymerase chain reaction conditions wherein coamplification of multiple target DNA sequences occurs in one or more cycles of identical melting, annealing and extending temperatures and times, wherein

each said X domain comprises a common sequence that is neither complementary to nor specific for said multiple target DNA sequences, whereby the synthesis of spurious amplification products are prevented; and

each said Y domain comprises a unique sequence, wherein said unique sequence is complementary to and specific for one of said multiple target DNA sequences suspected to be present in said DNA sample, whereby the synthesis of spurious amplification products are prevented; and, whereby a multiplicity of amplified target DNA sequences are obtained.